Photometrische Methoden:

UV/VIS Photometer

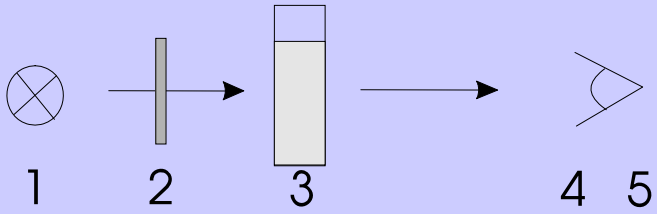
Grundlagen 1:

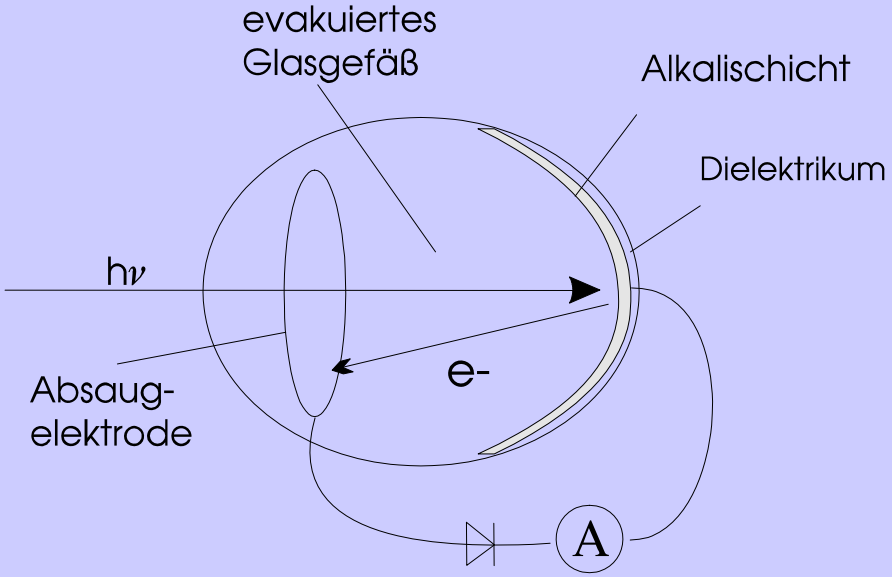
- Lichtmessung
- spezifische Lichtabsorption, Monochromasie
- Zusammenhang: Konzentration und Lichtschwächung das Lambert- Beersche Gesetz

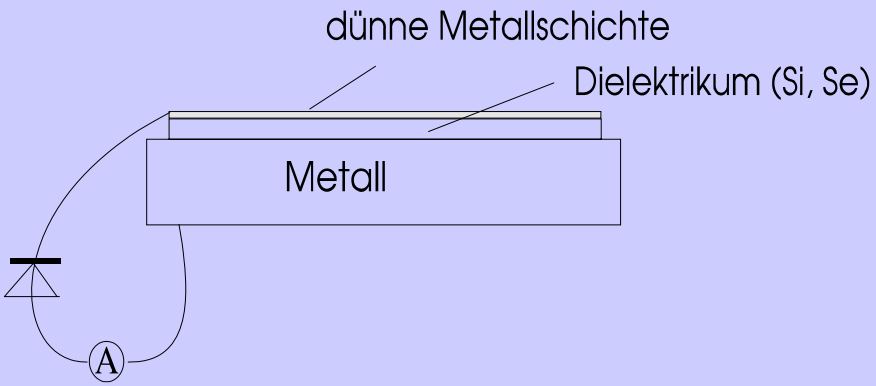
Messpraxis:

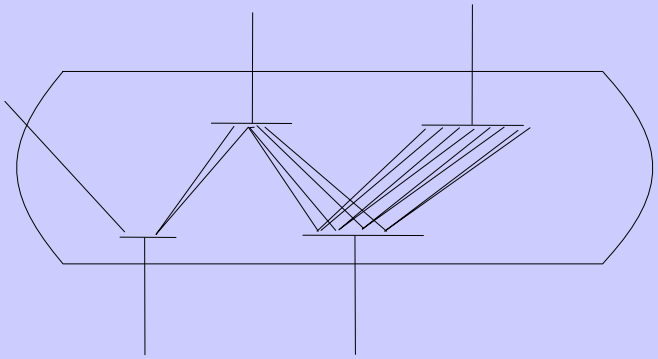
- Konzentrationsmessung (Mikroplatte), Kalibration
- Absorptionsspektrum (Diondenarray)
- Enzymkinetik (Zweistrahlphotometer)

Lichtmessung



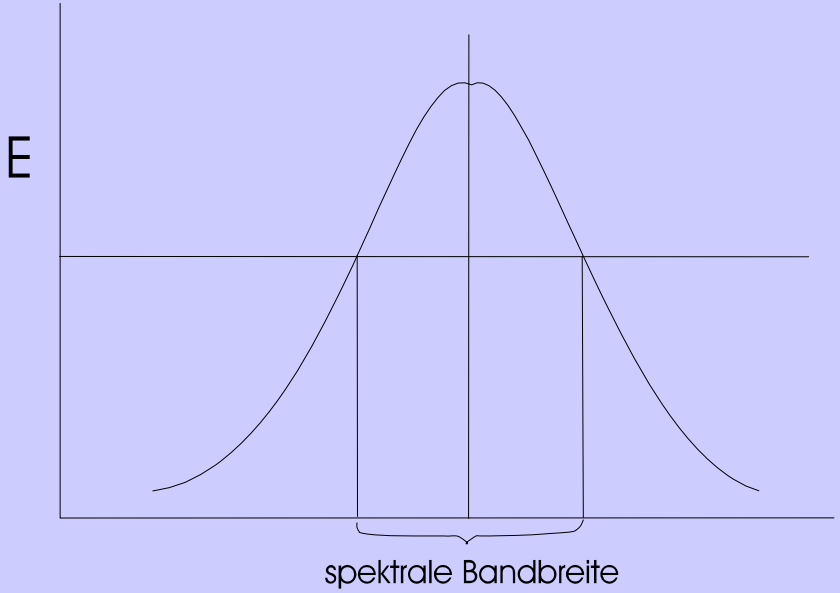


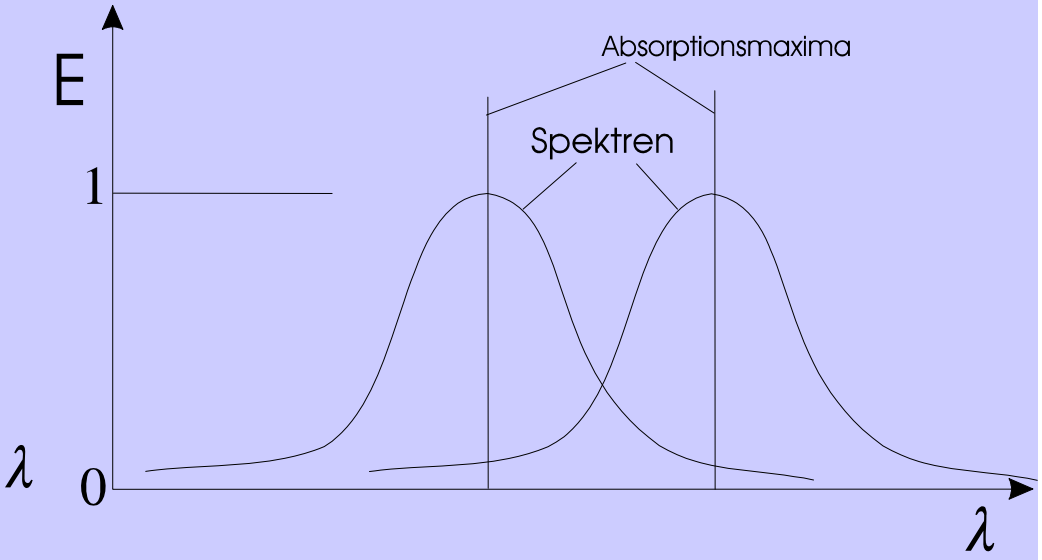


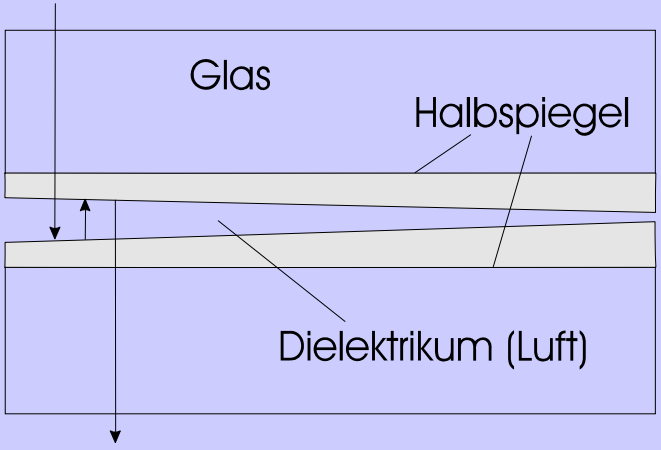


Sekundärelektronenvervielfacher - photomultiplier

Monochromatisches Licht

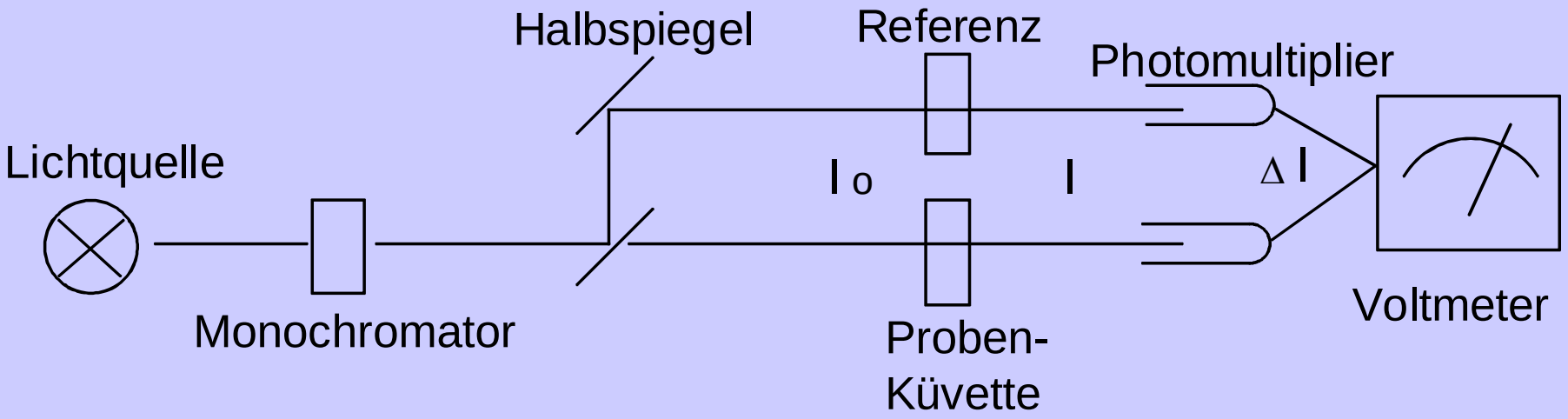






Interferenzverlauffilter

Photometrie



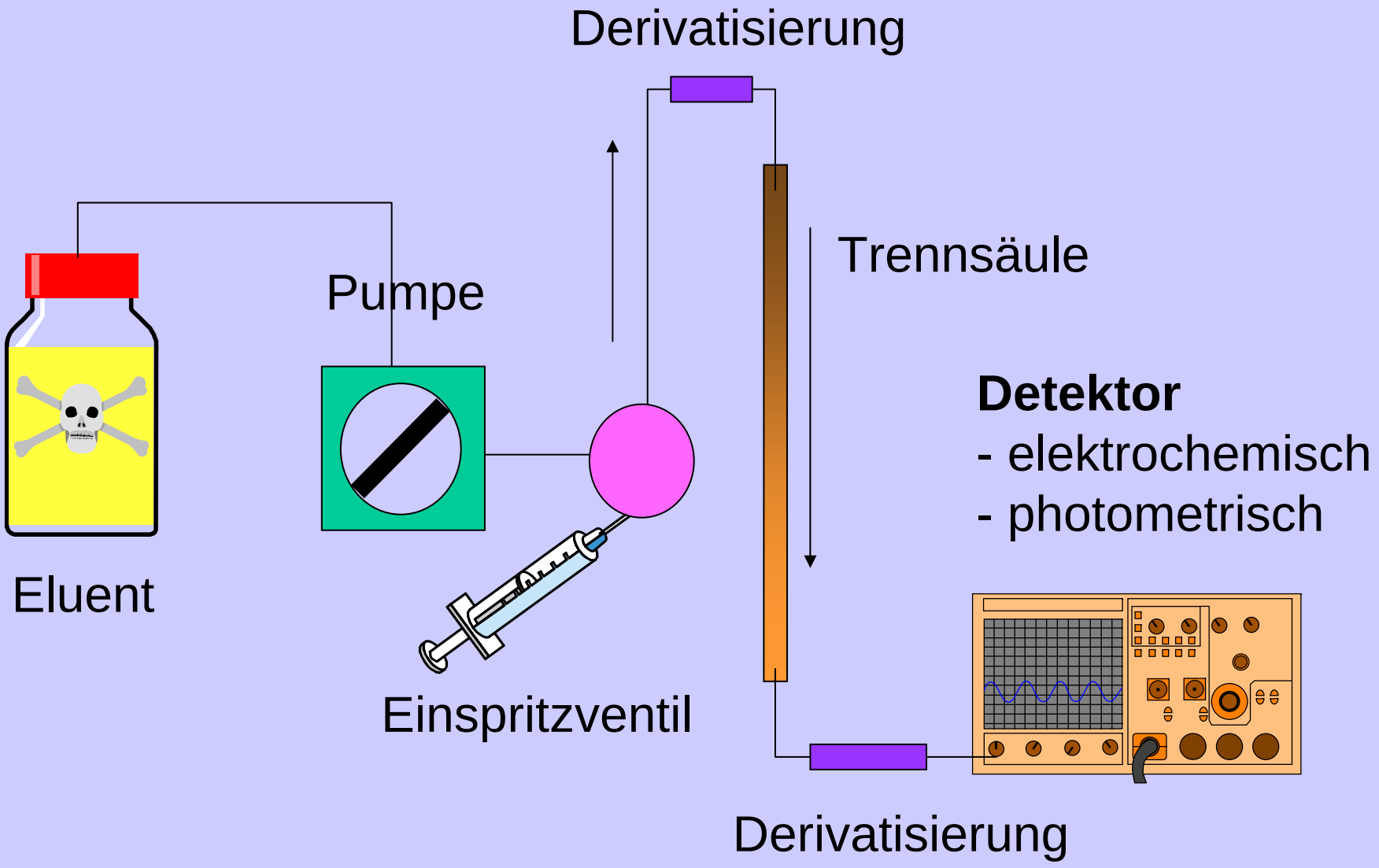
I_0,I ...Intensität
E ...Extinktion bzw. A ...Absorption
ε_λ ...molarer Extinktions- bzw. Absorptionskoeffizient
d ...Schichtdicke der Küvette
c ...Konzentration

$$A = \lg \frac{I_o}{I} = \varepsilon_\lambda \, c \, d$$ ...*Lambert und Beer*

cProbe / aProbe = cRef / a Ref

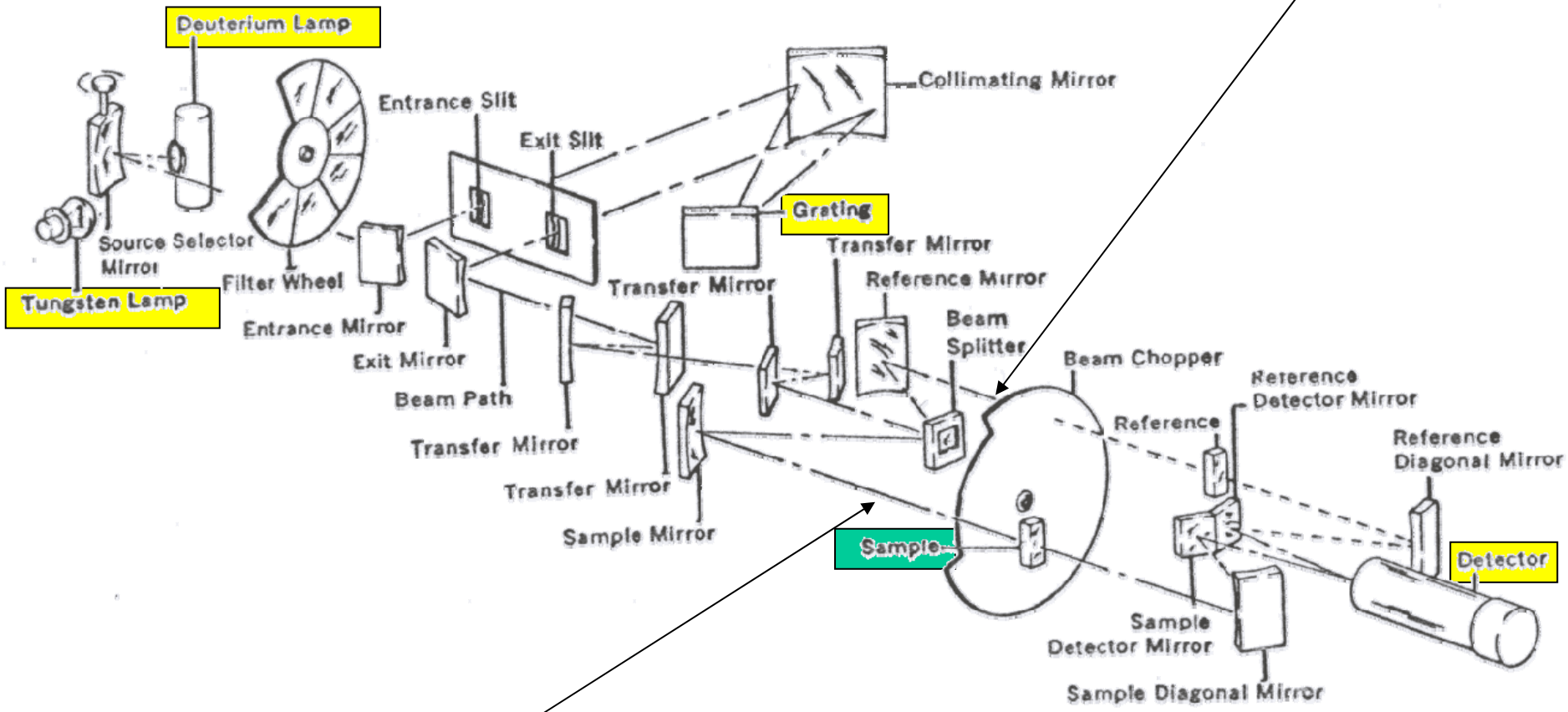
Wiederholung

Flüssigkeitschromatographie



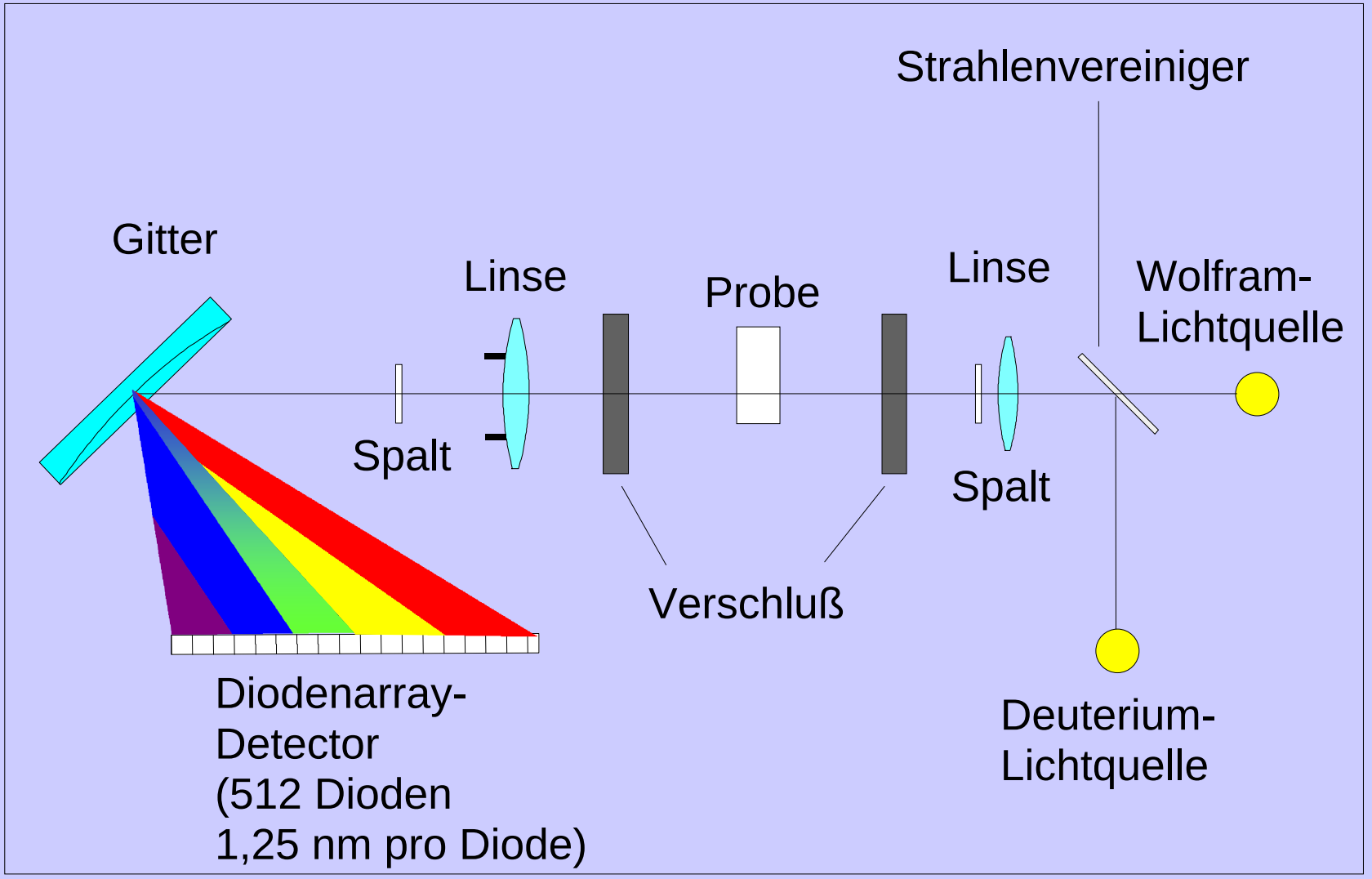
Zweistrahlphotometer:

Referenzstrahlengang

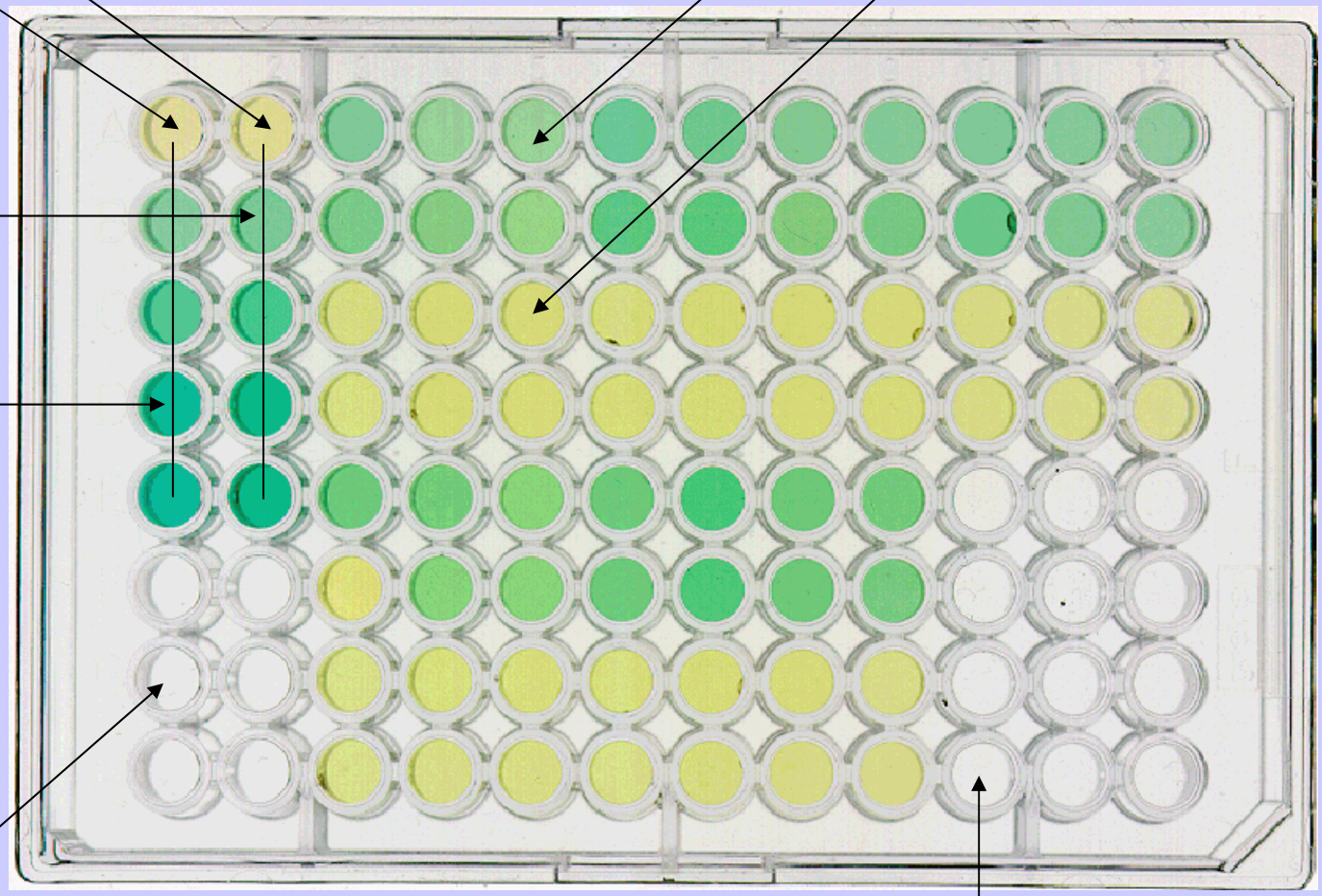


Probenstrahlengang

DIODENARRAY- SPEKTRALPHOTOMETER



Mikroplattenphotometer



Auswertung (Rechnung) über Kalibrationskurve

Position	Sample ID	Abs	Conc
A 1	Kalibrationspunkt 1	1	2
B 1	Kalibrationspunkt 2	2	3
C 1	Kalibrationspunkt 3	3	5
D 1	Kalibrationspunkt 4	4	7
E 1	Kalibrationspunkt 5	5	9
F 1			
G 1			
H 1			

→

y´ = kx + d

k = 1,80

d = -0,2

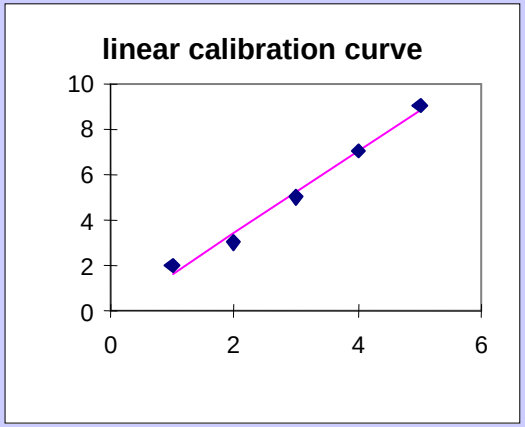
r = 0,9938837

Factor	Abs	Conc
1	1	**1,6**

Copy this formula to column D

to calculate the y´ values!

→



y'
1,6
3,4
5,2
7
8,8
-0,2
-0,2
-0,2

Wiederholung

Kalibrationskurve